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Insulin resistance in uremia mediated by postbinding defects

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Insulin resistance in uremia mediated by postbinding defects. Uremic subjects exhibit reduced glucose uptake in response to physiological increments in plasma insulin concentration. The relative contributions of insulin binding versus postbinding defects to this insulin resistance are not known. Because insulin normally requires only a small portion of its receptors to exert maximal biological effects on glucose uptake, it should be possible to overcome the insulin resistance of uremia, if it is due to a binding defect, by utilizing sufficiently high insulin levels to draw on the large reserve of spare receptors. If postbinding defects are present, however, then a decreased insulin response would be expected at high insulin concentrations. To determine whether or not the insulin resistance of uremia could be overcome by very high insulin levels, the dose-response relationship between plasma insulin concentration and insulin-mediated glucose uptake in seven chronically uremic and nine control subjects was evaluated. Insulin was infused at rates of 20, 40, 200, and 400 mU/min·m^2, and the plasma glucose concentration was held constant at the basal level by a variable glucose infusion (euglycemic insulin clamp technique). Under these steady state conditions of euglycemia, the glucose infusion rate plus endogenous glucose production (measured with ^{3}H-3-glucose) provides a measure of the amount of glucose taken up by the entire body. The steady state plasma insulin concentrations during the four insulin infusion protocols in the uremic subjects were consistently higher than in the controls. Despite the higher insulin levels in the uremics, the total amount of glucose taken up by the body was reduced by nearly 50% at each insulin plateau compared to controls. These results indicate that the in vivo dose-response curve relating plasma insulin concentration to glucose metabolism was shifted to the right in uremics and failed to normalize at high insulin levels. Basal hepatic glucose production was similar in uremic and control subjects. During the 20 and 40 mU/min·m^2 insulin clamp studies, suppression of hepatic glucose production in uremics (83 and 94%, respectively) was similar to controls (88 and 98%, respectively). Specific ^{125}I-insulin binding to circulating monocytes was measured in nine uremic and ten control subjects. No difference in insulin binding between the uremics and controls was observed. In normals, insulin binding correlated directly with glucose uptake and inversely with the fasting plasma insulin concentration. In uremics, no correlation was observed between insulin binding and glucose metabolism or between insulin binding and fasting plasma insulin concentration. It is concluded that in chronic renal failure insulin resistance is caused primarily by a postbinding defect in insulin action.

La résistance à l'insuline dans l'urémie médiée par des anomalies survenant après la liaison. Les urémiques ont une diminution de la captation du glucose en réponse à des augmentations physiologiques de la concentration plasmatique d'insuline. On ne connaît pas le rôle relatif des anomalies de la liaison de l'insuline et de celles survenant après la liaison dans cette résistance à l'insuline. Puisque, normalement, l'insuline n'a besoin que d'un petit nombre de récepteurs pour avoir ses effets biologiques maximum sur la captation du glucose, il devrait être possible de dépasser cette résistance à l'insuline d l'urémie, si elle était due à un défaut de liaison, en utilisant des concentrations d'insuline suffisamment élevées pour faire appel aux grandes quantités de récepteurs de réserve. Au contraire, si des anomalies après la liaison existent, on pourrait s'attendre à une diminution de la réponse insulinique pour de fortes concentrations d'insuline. Afin de déterminer si la résistance insulinique de l'urémie peut être dépassée à de très fortes concentrations d'insuline, la relation effet-dose entre la concentration plasmatique d'insuline et la captation du glucose dépendant de l'insuline chez sept urémiques chroniques et neuf sujets contrôles était étudié. L'insuline était perfusée à des débits de 20, 40, 200 et 400 mU/min·m^2, et la glycémie était maintenue constante à sa valeur de base par une perfusion de glucose à débit variable (technique du clamp insulinique euglycémique). Dans ces conditions à l'équilibre euglycémique, le débit de perfusion de glucose plus la production de glucose endogène (mesurée avec du ^{3}H-3-glucose) fournit une mesure de la quantité de glucose utilisée par l'organisme entier. Les concentrations plasmatiques d'insuline à l'état d'équilibre pendant les quatre protocoles de perfusion insulinique chez les urémiques étaient significativement plus hautes que chez les contrôles. Malgré les plus fortes concentrations d'insuline chez les urémiques, la quantité totale de glucose retenue par l'organisme était diminuée d'environ 50% à chaque plateau insulinique, par rapport aux contrôles. Ces résultats indiquent que la courbe dose-réponse in vivo entre la concentration plasmatique d'insuline et le métabolisme du glucose était décalée vers la droite chez les urémiques, et ne s'est pas normalisée à de fortes concentrations d'insuline. La production hépatique de base de glucose était identique chez les urémiques et les contrôles. Au cours des études avec des débits d'insuline de 20 et 40 mU/min·m^2, la suppression de la production hépatique de glucose chez les urémiques était identique à celle des contrôles. La liaison spécifique de ^{125}I insuline à des monocytes circulants a été mesurée chez neuf urémiques et dix contrôles. Il n'a pas été observé de différence entre la liaison de l'insuline chez les urémiques et chez les contrôles. Chez les normaux, la liaison insulinique était directement corrélée avec l'entrée du glucose, et inversement corrélée avec la concentration plasmatique d'insuline à jeûn. Chez les urémiques, nous n'avons observé aucune corrélation entre la liaison de l'insuline et le métabolisme du glucose ni entre la liaison de l'insuline et la concentration plasmatique d'insuline à jeûn. Il est conclu qu'au cours de l'insuffisance rénale chronique, la résistance à l'insuline est principalement due à un défaut de l'action de l'insuline en aval de sa liaison.

Numerous studies performed over the past two decades have established that insulin resistance is the major cause of the glucose intolerance of uremia. The presence of elevated basal concentrations of plasma immunoreactive insulin in uremic subjects [1–5], the blunted response to insulin in forearm perfusion studies [6], and the delayed and diminished hypoglycemic response after exogenous insulin [1, 5, 7–12], and tolbutamide [5, 7, 8, 11–13] provide evidence in support of this concept.

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More recent studies employing the euglycemic insulin clamp technique have extended and clarified these observations [14]. It was observed that the endogenous insulin secretory response to intravenous glucose was generally normal or increased, whereas total body glucose uptake in response to both endogenously secreted and intravenously infused insulin was uniformly diminished in uremic subjects. This reduced insulin responsiveness was shown subsequently to reside primarily in peripheral tissues, most likely skeletal muscle [15].

Little information exists concerning the mechanism(s) of this insulin resistance. Insulin, like other peptide hormones, is thought to initiate its effects on target tissues by binding to specific surface receptors [16]. The resulting hormone-receptor complex triggers a sequence of incompletely characterized membrane and intracellular events which produce characteristic biological responses. Thus, to evaluate the pathogenesis of insulin resistance, it is necessary to evaluate both the insulin binding as well as the postbinding steps.

Only a few studies of insulin binding in uremia have been reported, and these are conflicting. Insulin binding to erythrocytes from chronically uremic, nondialyzed subjects was found to be reduced [17]. After patients experienced hemodialysis therapy for 1 year [18], erythrocyte binding was reported to be increased above normal. In contrast, insulin binding to adipocytes from uremic rats was not altered [19]. At present, it is not possible to determine whether or not these differences are accounted for by differences in the methods employed in each study, by species variability, by the severity of uremia (and its treatment), or by variation in insulin binding among different tissues (that is, erythrocytes versus adipocytes).

Direct measurements of postbinding events in human muscle, the tissue primarily responsible for glucose metabolism when glucose and insulin are infused intravenously, are not possible in vivo. However, inferences about their activity are possible because of the distinctive relationship between insulin binding and insulin action. Simultaneous measurements of insulin binding and insulin effects (for example, glucose transport) in studies of muscle [20] and fat [21] in vitro have demonstrated that insulin bioactivity increases rapidly with insulin concentration within the physiological range; however, bioactivity reaches a plateau when only a small portion of the total number of receptors is occupied, usually 5 to 20% [20, 21]. The unoccupied, spare receptors appear to be identical to their occupied counterparts, and the sequence in which receptors are recruited is determined randomly [21]. Thus, at low hormone concentrations, receptor occupancy is rate controlling for glucose transport. However, at approximately 5 to 20% occupancy, postbinding steps become saturated and thereby rate limiting [22–24]. According to this scheme, in circumstances in which alterations in insulin binding alone are responsible for insulin resistance, the dose-response curve relating insulin concentration to glucose uptake is shifted to the right; however, the maximal hormone responsiveness is unaltered because the high insulin concentrations will recruit sufficient spare receptors to normalize the response. When postbinding abnormalities are present, no amount of hormone is able to produce normal maximal responsiveness. Although this is a simplification of a very complex process, it does provide a framework which may be helpful in distinguishing between binding versus postbinding defects in insulin action.

To examine the contribution of altered insulin binding versus postbinding defects in the insulin resistance of uremia, we constructed in vivo dose-response curves relating total body insulin-mediated glucose metabolism to the plasma insulin concentration in seven chronically uremic subjects. In addition, we correlated insulin-mediated glucose metabolism with measures of total, specific ^{125}I-insulin binding to monocytes.

Methods

Subjects. Seven adult, ambulatory patients with advanced, chronic renal failure served as the study population for the euglycemic insulin clamp studies. All experiments were performed in the Clinical Research Center of Yale-New Haven Hospital. Diagnoses and relevant clinical and biochemical data are presented in Table 1. All patients were within 20% of ideal body weight (Metropolitan Life Insurance Tables, 1959). None had a family history of diabetes mellitus, or clinical evidence of diabetic retinopathy or neuropathy. All were edema free at the time of study. Excluding one patient with chronic obstructive lung disease, none had significant extrarenal disease. Blood chemistries were normal or only slightly altered (Table 1).

Except for sodium bicarbonate and aluminum hydroxide, no patients were taking any medications for at least 3 days prior to study. All subjects were free of gastrointestinal symptoms and were consuming a weight maintaining diet containing at least 250 g of carbohydrate for 3 days prior to study. No subjects were restricted in their protein intake.

Nine healthy volunteers of normal weight (four women and five men) served as control subjects. Their ages ranged from 23 to 54 years (mean = 38 ± 4 years). Their obesity index (ratio of observed to ideal body weight) was 0.97 to 1.18 (mean = 1.05 ± 0.03). The purpose and potential risks of the study were explained to all subjects, and their written, voluntary consent was obtained prior to their participation. The study protocol was approved by the Human Investigations Committee of Yale University School of Medicine. All tests were performed in the postabsorptive state beginning at 8 A.M. following a 10-hr overnight fast.

Euglycemic insulin clamp. A polyethylene catheter was inserted into an antecubital vein for infusion of insulin and glucose. A second catheter was placed in a retrograde direction in a wrist vein for blood sampling, and that hand was kept in a heating box at 70° C to arterialize the venous blood [25]. Following collection of at least three baseline samples, a primed-continuous infusion of crystalline porcine insulin (Eli Lilly Co., Indianapolis, Indiana) was administered to raise acutely and maintain the arterial plasma insulin concentration at the desired level. The plasma glucose concentration was kept at the basal level by determination of plasma glucose every 5 min and appropriate adjustment of the infusion rate of a 20% glucose solution as previously described [26]. Under these steady state conditions of euglycemia, glucose input equals glucose utilization. Glucose input is comprised of the exogenous infusion required to maintain euglycemia, and the endogenous glucose production rate (see below); their sum thus serves as a measure of the body's sensitivity to the infused insulin [26].

Each subject received a maximum of four insulin clamp studies performed at approximately 1-week intervals to construct a dose-response curve relating whole body sensitivity to insulin to the plasma insulin concentration. The insulin infusion

Table 1. Summary of clinical and laboratory data

Patient no.	Age *year*	Sex	Ht *cm*	Wt *kg*	Obesity index	Diagnosis	Duration of renal failure[a] *year*	C_{Cr} *ml/min*	Serum Urea nitrogen *mg/dl*	Serum Creatinine *mg/dl*	Serum Phosphate *mg/dl*	Serum HCO_3^- *mEq/liter*	Serum K^+ *mEq/liter*	Blood pH
1	50	M	177.8	79.5	1.17	Accelerated nephrosclerosis	2	9	95	9.4	5.2	24	4.8	7.42
2	49	M	177.8	67.0	0.98	Chronic glomerulonephritis	5	24	69	3.5	4.4	22	4.5	7.38
3	56	M	172.7	76.8	1.19	Chronic glomerulonephritis	9	15	55	5.9	5.2	19	5.1	7.40
4	57	M	170.2	57.7	0.92	Chronic glomerulonephritis	10	18	44	3.8	3.3	18	3.9	7.35
5	53	F	166.4	55.0	0.92	Membranous glomerulopathy	12	11	106	4.6	5.7	25	3.9	7.43
6	42	M	180.2	84.2	1.20	Chronic glomerulonephritis	3	15	96	6.5	5.0	19	4.7	7.35
7	19	M	177.8	63.2	0.93	Obstructive nephropathy	10	7	92	15.9	6.5	20	4.2	7.33
Mean	46		174.7	69.1	1.04		7	14	80	7.1	5.0	21	4.4	7.38
SEM	5		1.9	4.3	0.05		2	2	9	1.7	0.4	1	0.2	0.01

[a] From the first known elevation in serum urea nitrogen and/or serum creatinine.

rate was varied over a 20-fold range (20, 40, 200, and 400 mU/min·m^2) to achieve increments in plasma insulin of approximately 50, 100, 500, and 1000 μU/ml. In the uremic group it was feasible to perform 25 of the 28 desired studies; in the controls, 31 of 36. In each group, at least five subjects were studied at each of the four insulin infusion rates.

Endogenous glucose production. The two lower dose clamp studies were performed in conjunction with tritiated glucose to measure the effect of hyperinsulinemia on hepatic glucose production, as previously described [27]. For 120 min in the control group and 150 min in the uremic group, each subject's glucose pool was labeled by a primed-continuous infusion of ^{3}H-3-glucose (New England Nuclear, Boston, Massachusetts). The labeled glucose was administered as a 25 μCi bolus followed by a continuous infusion at the rate of 0.25 μCi/min. Plasma samples for the determination of glucose specific activity were taken at −60 and −30 min of this equilibration period, and then every 5 min until time 0 when the insulin infusion was begun. A steady state of glucose specific activity was achieved by this method in each study, and the specific activity plateau was used to calculate glucose appearance in the basal state. Because glucose production by nonhepatic tissues in the fasting state is negligible, the rate of basal glucose appearance equals hepatic glucose production. After this equilibration period, the insulin clamp was begun and the tritiated glucose infusion continued at the same rate. During the insulin clamp, plasma samples for glucose specific activity were obtained at 15-min intervals for the first 90 min, and then every 5 min for the final 20 min of the 2-hr study.

Insulin binding to monocytes. Nine subjects of normal weight with chronic uremia (creatinine clearance = 15 ± 1 ml/min) gave blood samples for the determination of ^{125}I-insulin binding to monocytes. This group included three of the seven subjects who participated in the dose-response study, three subjects who received only 40 mU/min·m^2 insulin clamp, and three who declined insulin clamp studies. Their ages ranged from 41 to 63 years (mean = 52 ± 3 years). The obesity index was from 0.92 to 1.20 (mean = 1.09 ± 0.04). Ten healthy volunteers of normal weight served as controls, including six who also participated in the dose-response study. Their ages ranged from 25 to 54 years (mean = 42 ± 3 years). Their obesity index was 0.88 to 1.18 (mean = 1.02 ± 0.03).

Whole blood (100 ml) was drawn into heparinized syringes (20 U/ml blood) from each subject following a 10-hr overnight fast. The blood was diluted 1:1 with phosphate buffered saline, pH 7.4, layered onto Ficoll-Hypaque, and centrifuged as described by Boyum [28]. The mononuclear cell layer was removed, washed once in chilled phosphate buffered saline, and once in Hepes (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) lymphocyte buffer (Hepes 100 mM, sodium chloride 120 mM, potassium chloride 5 mM, EDTA 1 mM, glucose 9 mM, sodium acetate 15 mM, magnesium sulfate 1.3 mM, bovine serum albumin 10 g/liter, pH 8.0). The final cell pellet was diluted in Hepes buffer to a concentration of 35 to 45 × 10^6 cells/ml. Viability was assessed by trypan blue exclusion, and was always greater than 97%. For estimation of monocytes, 100 μl of the cell suspension, 10 μl of latex beads (10% solids, 0.8 μm diameter, Sigma, St. Louis, Missouri), and 0.5 ml of fetal calf serum were incubated for 40 min at 37° C in a shaking water bath. Cells containing four or more beads were counted as monocytes, and averaged 33 ± 3% in the uremic group and 20 ± 2% in the controls.

^{125}I-insulin was prepared at a specific activity of 150 to 180 μCi/μg by the chloramine-T method of Hunter and Greenwood [29], as modified by Freychet et al [30]. Purified porcine insulin for iodination was a gift of Lilly Research Laboratories, Indianapolis, Indiana, and sodium ^{125}I was purchased from ICN (Irvine, California). ^{125}I-insulin (0.1 to 0.2 ng/ml) was incubated with 0.4 ml of cell suspension with or without cold insulin in a final incubation volume of 0.5 ml. The range of total insulin

Table 2. Plasma insulin concentrations and glucose metabolism in the basal state and during euglycemic hyperinsulinemia (insulin clamp)[a]

	Basal		Insulin clamp							
			20 mU/min·m²		40 mU/min·m²		200 mU/min·m²		400 mU/min·m²	
	Fasting plasma insulin μU/ml	Glucose metabolism[b] mg/min·kg	SSPI[c] μU/ml	M[d] mg/min·kg	SSPI μU/ml	M mg/min·kg	SSPI μU/ml	M mg/min·kg	SSPI μU/ml	M mg/min·kg
Controls	14 ± 1	2.2 ± 0.1	69 ± 4	5.2 ± 0.4	109 ± 7	6.9 ± 0.5	741 ± 50	11.1 ± 0.8	1880 ± 182	11.5 ± 0.6
Uremics	29 ± 4[e]	2.1 ± 0.1	84 ± 12	2.7 ± 0.2[e]	147 ± 13[f]	3.9 ± 0.5[e]	1118 ± 127[f]	6.5 ± 0.7[e]	3181 ± 367[f]	7.0 ± 0.6[e]

[a] All values are mean ± SEM.
[b] Measured with ^{3}H-3-glucose.
[c] Steady state plasma insulin concentration during the 20- to 120-min period.
[d] Mean rate of glucose uptake during the 20- to 120-min period.
[e] $P < 0.01$ compared to controls.
[f] $P < 0.05$ compared to controls.

concentrations varied from 0.1 to 100,000 ng/ml. The incubation was carried out in Hepes buffer for 180 min at 22° C. After the incubation, duplicate 0.2-ml aliquots of the cell suspension were transferred to microfuge tubes containing 0.1 ml of chilled Hepes buffer, and centrifuged at 10,000 RPM for 2 min in a Beckman Microfuge (Beckman Instruments, Inc., Fullerton, California). The supernatant was removed by aspiration, and the radioactivity in the cell pellet was counted in a gamma counter. Nonspecific binding, as defined by the amount of ^{125}I-insulin bound to the cell pellet in the presence of 10^5 ng/ml insulin, was 0.3 to 0.5% of total radioactivity for both normal and uremic subjects. The nonspecific binding was subtracted from total binding to give the specific binding, and this was normalized to 10^7 monocytes/ml to facilitate comparisons. Degradation of tracer by the incubation medium, as assessed by ^{125}I solubility in 10% trichloroacetic acid, was always less than 10% following the 3-hr incubation.

Analytical procedures. Plasma glucose was determined by the glucose oxidase method (Glucostat, Beckman Instruments, Inc., Fullerton, California). Methods for the determinations of plasma immunoreactive insulin [31] and the specific activity of plasma glucose [32] have been published previously. The inter- and intra-assay variability for insulin determination was 10.2% and 6.2%, respectively.

Calculations. For the euglycemic insulin clamp studies, the glucose infusion rate was averaged over the final 20- to 120-min time period. The total amount of glucose metabolized by the entire body (M) was calculated by adding the rate of endogenous glucose production (see below) to the exogenous glucose infusion rate required to maintain euglycemia. Steady state plasma glucose and insulin levels were determined from the mean values for the 20- to 120-min time period.

Glucose turnover in the basal state was determined by dividing the tritiated glucose infusion rate (cpm/min) by the steady state glucose specific activity (cpm/mg) achieved during the final 30 min of the tracer equilibration period. After the insulin infusion is begun, a nonsteady state condition for glucose specific activity exists. The total rate of appearance of glucose in the systemic circulation was then calculated from Steele's equations in their derivative form [33], using a value of 0.65 for the pool fraction [34]. The rate of endogenous (hepatic) glucose production was estimated by subtracting the exogenous infusion rate from the total appearance rate as calculated by the isotopic tracer technique.

^{125}I-insulin binding data were analyzed by three methods: *(1)* competition curve, relating percent ^{125}I-insulin specifically bound versus total (hot plus cold) insulin concentration; *(2)* Scatchard plot, relating bound/free insulin versus bound insulin [35]; and *(3)* average affinity profile method of De Meyts and Roth, relating average insulin affinity ($\bar{K}_i$) for its receptor versus fractional receptor occupancy [36].

All data are presented as mean ± SEM except where indicated otherwise. All comparisons of mean values between groups were calculated by unpaired *t* test analysis, and within groups by paired *t* test analysis. Coefficients of correlation were determined by standard procedures.

Results

Euglycemic insulin clamp. The basal glucose concentration was similar in the uremic (94 ± 4 mg/dl) and the control subjects (92 ± 2 mg/dl). In the 25 euglycemic insulin clamp studies performed in uremic subjects, the plasma glucose was maintained at a steady state level of 94 ± 2 mg/dl, with an average coefficient of variation of 4.0 ± 0.4%; in 31 studies performed in the control group, the corresponding values were 91 ± 1 mg/dl and 4.6 ± 0.3%.

The basal plasma insulin concentration was higher in the uremics than controls (29 ± 4 μU/ml versus 14 ± 1 μU/ml, $P < 0.01$). The steady state insulin concentration from 20 to 120 min was also higher in the uremic group at each of the four insulin infusion rates, although the difference was not significant in the lowest dose study (Table 2). The stability of the plasma insulin plateau is indicated by a coefficient of variation of 13.6 ± 1.1% in the uremic group, and 14.4 ± 1.4% in the control group.

The total amount of glucose metabolized during each of the four insulin clamp studies is shown in Table 2. The average rate of glucose utilization was reduced by approximately 50% in all four insulin infusion protocols. Figure 1 shows the dose-response relationship between the plasma insulin concentration and insulin-mediated glucose metabolism. The first point on each curve represents the amount of fasting glucose metabolism that is insulin-dependent. This has been approximated to represent 30% of basal hepatic glucose production [37]. The remaining 70% of basal glucose metabolism, 1.49 ± 0.04 mg/min·kg in the normals and 1.50 ± 0.11 mg/min·kg in the uremics, represents insulin-independent glucose metabolism and has been subtracted from the total glucose metabolized during the euglycemic insulin clamp studies. Because the 70% of the basal

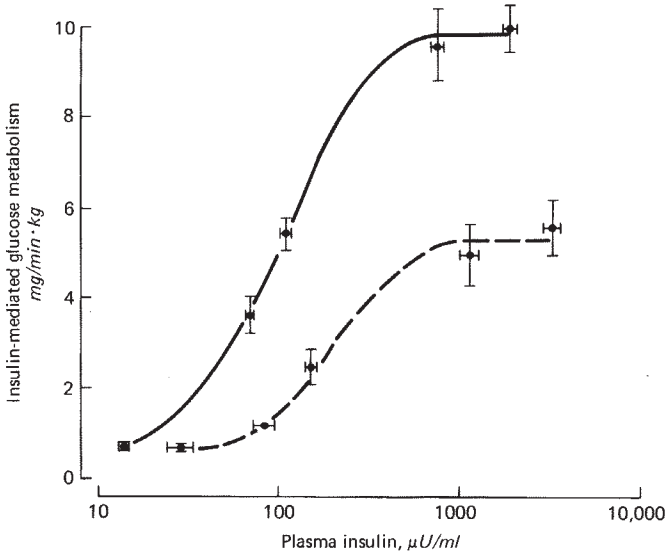

Fig. 1. *Dose-response relationship between the plasma insulin concentration and insulin-mediated glucose metabolism in seven uremic* (●---●) *and nine control* (●——●) *subjects.* Insulin-dependent glucose metabolism in the basal state (first *point* on each *curve*) was assumed to be 30% of endogenous glucose production as determined from ^{3}H-3-glucose kinetics.

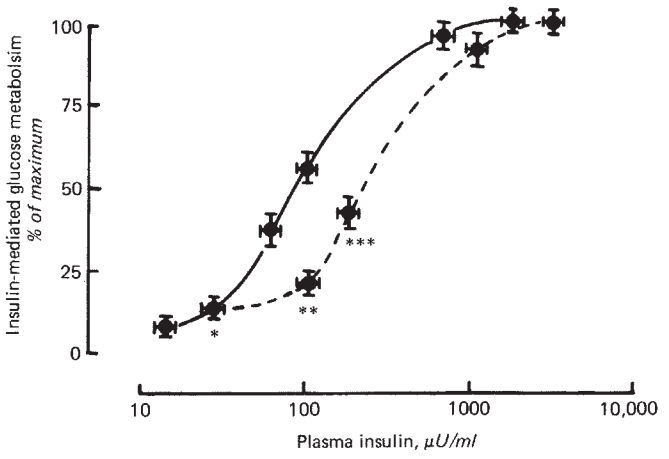

Fig. 2. *Dose-response relationship between the plasma insulin concentration and percent of maximal glucose metabolism obtained in the 400 mU/min·m^2 euglycemic insulin clamp.* Data and symbols are identical to those in Figure 1. *Asterisks* denote significant difference between uremic and respective control studies (*$P < 0.005$, **$P < 0.025$, ***$P < 0.05$).

glucose metabolism which is insulin-independent is determined only by glucose mass action, it would not be expected to change under the euglycemic conditions employed in the present studies. Although the 400 mU/min·m^2 insulin infusion rate produced slightly higher mean rates of glucose metabolism in each group in comparison to the 200 mU/min·m^2 insulin clamp (Fig. 1), these differences were not statistically significant. Thus, the responses in both groups appeared to achieve a plateau with the 400 mU/min·m^2 insulin clamp studies. Compared to normal, the dose-response curve for uremics had a distinctly lower plateau (5.3 versus 9.3 mg/min·kg, $P < 0.001$) and was shifted to the right. Consequently, the plasma insulin concentration required to achieve half-maximal stimulation of glucose metabolism was significantly higher in the uremics (187 ± 25 versus 96 ± 11 μU/ml, $P < 0.01$).

In Figure 2, the dose-response data of Table 2 are plotted as insulin concentration versus percent of the maximal insulin response (observed in the 400 mU/min·m^2 euglycemic insulin clamp). Expressed in this way, the curve for uremics again demonstrates a significant shift to the right.

Hepatic glucose production. In the basal state hepatic glucose production in the uremics (2.14 ± 0.10 mg/min·kg) was similar to that of controls (2.23 ± 0.06 mg/min·kg). In the 20 and 40 mU/min·m^2 euglycemic insulin clamp studies, glucose production by the liver suppressed by 83 and 94% in the uremics; the degree of suppression was not different from the corresponding control values, 88 and 98%. The time course of suppression over the 2-hr study was similar in both groups.

Monocyte binding. The competition curves for total, specific ^{125}I-insulin binding to monocytes is shown in Figure 3. Although five of the nine observations in uremics were outside the range of normals, the mean binding (5.9 ± 0.6%) was similar to the control value (6.2 ± 0.2%). The half-maximal binding occurred at identical insulin concentrations in the two groups (2.4 ng/ml). Scatchard analysis of the binding data revealed very similar curves with identical *x*-axis extrapolations (1.3 ng/ml). The total number of insulin receptors per cell was similar in uremic (11,000 ± 2000) and control groups (15,000 ± 2000). Average affinity profiles were also similar, with average affinity of the unoccupied receptor in the uremic group (3.2/10 nM) slightly but not significantly less than that of the control group (3.6/10 nM).

In normal subjects, total ^{125}I-insulin specifically bound to monocytes correlated directly ($r = 0.67$, $P < 0.05$) with the total glucose metabolism observed in the 40 mU/min·m^2 insulin clamp (Fig. 4). In contrast, there was no apparent relationship between these variables in uremic subjects. There was a significant inverse correlation between the fasting plasma insulin concentration and ^{125}I-insulin binding to monocytes in normal ($r = 0.76$, $P < 0.05$) but not in uremic subjects (Fig. 5).

Discussion

Under the conditions of euglycemic hyperinsulinemia employed in the present studies, the splanchnic bed of both normal and uremic subjects is known to utilize similar, small amounts of glucose [15, 38]. Also, suppression of hepatic glucose production is similar. Thus, by exclusion, the blunted response to exogenous insulin observed in our uremic patients (Fig. 1) must result from peripheral, that is, extra-splanchnic, resistance to the action of insulin. Moreover, because fat accounts for only a small percentage of insulin-mediated glucose uptake [39], the defect most likely resides predominantly in skeletal muscle. This conclusion is consistent with the original observation of Westervelt that stimulation of glucose uptake by insulin is attenuated in forearm perfusion studies of uremic patients [6]. Similar findings were subsequently reported with the hindlimb perfusion technique in uremic rats [40], and with leg catheterization in uremic patients [15]. It should be pointed out, however, that fat (as well as other tissues) could be severely resistant to the action of insulin but this would have little impact

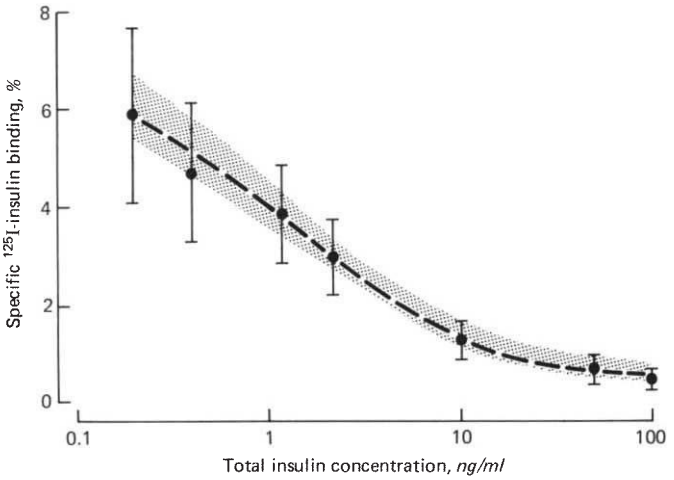

Fig. 3. *^{125}I-insulin specifically bound to monocytes versus total (hot plus cold) insulin concentration.* Incubations were performed in the absence (initial *point* on the *curve*) and presence of increasing concentrations of unlabeled insulin. *Shaded area* represents the data (mean ± SD) for 10 control subjects. For the uremic group (●---●), each *point* represents mean ± SD of nine subjects.

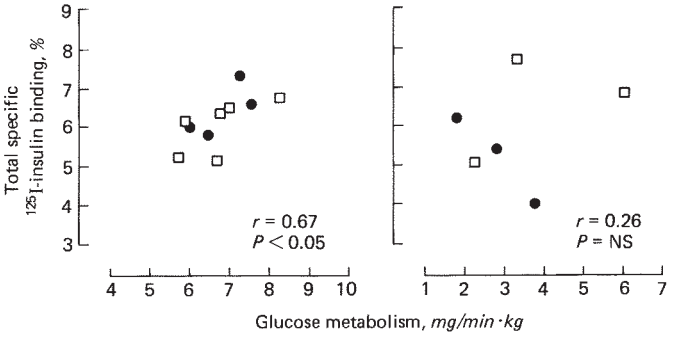

Fig. 4. *Relationship between ^{125}I-insulin specifically bound to monocytes and glucose metabolism (40 mU/min·m^2 insulin clamp) in control* (left panel) *and uremic* (right panel) *subjects who participated in the dose-response study* (□), *or who received only the 40 mU/min·m^2 insulin clamp* (●).

on glucose metabolism as measured with the euglycemic insulin clamp technique. In fact, recent studies in uremic rats have demonstrated specific defects in adipocyte glucose metabolism [19].

The cellular mechanism underlying this impairment in insulin action remains to be elucidated. The relationship between the plasma insulin concentration and glucose metabolism is complex. The determinants of this relationship include insulin binding, which in turn is conditioned by insulin receptor number and affinity; by postbinding activities of the receptor, such as aggregation, internalization, and degradation [41]; by the number and activity of glucose transport systems [42]; and by intracellular pathways for glucose disposal [43]. Furthermore, the insulin response in vivo represents the simultaneous activities of multiple insulin responsive tissues, which may be differentially affected by the hormone. Despite these complexities, it is possible to examine the mechanism(s) of insulin resistance in vivo because of the characteristic relationship between insulin binding and insulin action.

Stephenson proposed many years ago that the biological effects of hormonal stimulation might occur when less than the total number of cell surface receptors were bound by the hormone [24]. Subsequently, animal studies from many laboratories have confirmed this hypothesis. Both adipocytes [21, 44] and isolated soleus muscle [20, 45] achieve maximal glucose uptake when only a small fraction (5 to 20%) of surface insulin receptors are bound. Reduction in the number of receptors by trypsin exposure [21], spontaneous obesity [44], or experimental obesity [45] does not impair the maximal insulin response unless the number of receptors is decreased by more than 80 to 95% [21]. Similar observations have been made in human subjects. Kolterman et al have estimated that maximal rates of glucose disposal in normal subjects (measured by the euglycemic insulin clamp) are achieved when at most 40% of insulin receptors on adipocytes are occupied [46]. Thus, it would appear to be a general phenomenon that the majority of insulin receptors are unoccupied at hormone concentrations capable of eliciting a maximal functional response. Even though all of the receptors are presumably capable of normal function [47], at low fractional occupancy some postbinding activity becomes rate limiting for hormone action. Observations of this sort have led to the proposal that mechanisms for insulin resistance could be inferred from the shape and position of the dose-response curve for insulin action [48, 49]. An isolated defect in insulin binding would be expected to result in a rightward shift in the dose-response curve, with a normal response occurring at higher insulin concentrations, provided that the minimal number of receptors normally required for a maximal response still remains. In contrast, a postbinding defect would produce a decreased response to maximally effective insulin concentrations.

The data presented in this report demonstrate both a rightward shift as well as decreased maximal responsiveness in the dose-response curve relating plasma insulin concentration to glucose metabolism in uremic subjects. Because insulin binding was normal, these results are most compatible with an isolated postbinding defect in insulin-mediated glucose metabolism. It is important, however, to keep in mind the assumptions behind this conclusion. During the insulin clamp study, the major site of glucose uptake is muscle. It must be assumed, therefore, that spare receptors exist in human muscle as they do in rodent muscle [20] and human adipocytes [46]. It also must be assumed that a postbinding defect cannot be overcome at high insulin concentrations. Although this appears to be the case in rodent obesity for both adipocytes [44] and skeletal muscle [20, 45], it has not been established for either tissue in man.

It is also of interest to examine the data relating the plasma insulin concentration and insulin-mediated glucose metabolism in terms of the percent of maximal response. Kolterman et al have suggested that if the biological response data are normalized to the percent of maximum, postbinding contributions to altered glucose utilization may be eliminated [46]. Consequently, this mode of data expression should reveal directly any contribution of impaired insulin binding to the insulin resistance. For a simple system involving a single tissue with a single rate limiting intracellular step, if one plots the dose-response

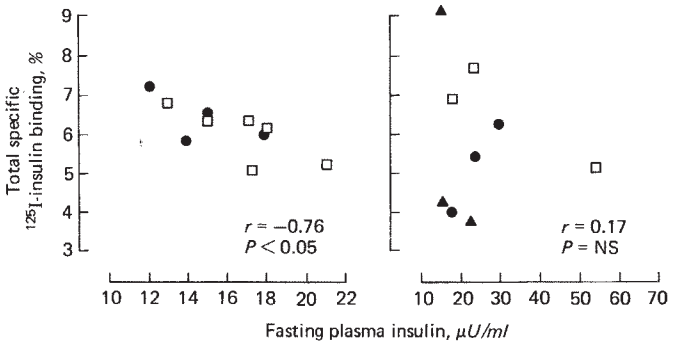

Fig. 5. *Relationship between ^{125}I-insulin specifically bound to monocytes and fasting plasma insulin concentration in control* (left panel) *and uremic* (right panel) *subjects.* Symbols are the same as those in Figure 4. Three subjects (▲) did not have insulin clamp studies.

data in this way (percent of maximal response) an isolated postbinding defect would be manifested by a normal curve. However, in man in vivo where multiple tissues and processes (that is, muscle, adipose, hepatic, extrahepatic splanchnic glucose uptake, and suppression of hepatic glucose production) contribute to the overall rate of glucose utilization and where multiple intracellular steps may be rate limiting, the ascending or steep portion of the insulin dose-response curve may be difficult to interpret and a rightward shift may or may not be present. Figure 2 depicts a plot of the insulin concentration versus the percent of maximum response for our uremic subjects; a significant shift to the right for the uremics was observed. Because insulin binding was normal, these results suggest that postbinding defects in insulin action in man may also be accompanied by a rightward shift in the percent maximum dose-response curve, so that caution must be used in interpreting this complex relationship.

Of the six subjects who received both an insulin binding study and a 40 mU/min·m^2 insulin clamp study, all demonstrated significant reductions in insulin-mediated glucose metabolism, while only one had decreased insulin binding. It should be noted, however, that the insulin binding studies were carried out on circulating monocytes. It is known that this cell type does not demonstrate the full range of biologic responses to insulin. Furthermore, the incubation conditions (pH, temperature and buffer), designed to optimize binding and minimize degradation of the tracer, are unphysiologic. Accordingly, whether or not monocyte binding accurately reflects binding in conventional insulin target tissues in vivo can be argued. Nevertheless, considerable evidence suggests that they are well correlated. For example, Gavin et al have shown that the insulin binding properties of the human monocyte are very similar to those of liver and fat [50]. Soll et al demonstrated that insulin binding to thymic lymphocytes from obese mice parallels insulin binding alterations in hepatocytes and adipocytes [51]. More recently Olefsky reported similar insulin binding changes in monocytes and adipocytes from obese patients [52]. In the present investigation, however, insulin-mediated glucose metabolism is determined primarily by the hormone's effects on two tissues, liver and skeletal muscle. To our knowledge, no one has reported data on insulin binding to human muscle or liver and correlated this with insulin binding to monocytes. Until such data are available, the present insulin binding studies should be interpreted cautiously.

With the above reservations in mind, it is interesting to examine the correlation between insulin binding and insulin action observed in these studies. During the 40 mU/min·m^2 insulin clamp study, insulin-mediated glucose metabolism in the control group correlated directly with monocyte binding at tracer insulin concentration (Fig. 4). In contrast, there was no correlation between these measurements in the uremic group. A similar dissociation between insulin binding and insulin action has been demonstrated previously in such diverse circumstances as high carbohydrate feeding [53], starvation [54], and noninsulin-dependent diabetes mellitus [55]. Chronic renal failure appears to be another condition in which the normal relationship between insulin binding and responsiveness can be disrupted.

Many previous studies have demonstrated a negative correlation between the fasting plasma insulin concentration and insulin binding to its receptor, fostering the current view that the basal insulin level is the major factor responsible for regulating its receptor concentration [41]. In agreement with this hypothesis, an inverse relationship ($r = -0.76$, $P < 0.05$) between insulin binding to monocytes and the fasting plasma insulin concentration was observed in our control subjects (Fig. 5). In contrast, no correlation between these variables was observed in the uremic group. Despite fasting insulin levels that were twice those in the controls (29 versus 14 μU/ml), mean insulin binding was not diminished in the uremics. Even in the two subjects with the highest fasting insulin levels (52 and 30 μU/ml), no decrease in monocyte binding was observed (5.1 and 6.2%, respectively). Our results seem to indicate that in many uremic individuals, factors in addition to insulin play a role in regulating receptor activity. In this regard, a recent report by Caro and Amatruda indicates that the ability of insulin to regulate receptor activity is mediated by postreceptor events [56]. These authors suggested that the presence of normal insulin binding in some insulin resistance states may result from resistance to this regulatory effect of insulin on its own receptor concentration. Alternatively, normal binding in insulin-resistant states may represent a compensatory process by as yet undefined mechanisms.

In previous studies of uremic subjects which employed tritiated glucose in conjunction with the hepatic venous catheterization technique, we have shown that elevation of the plasma insulin concentration by approximately 100 μU/ml inhibited hepatic glucose production normally [15]. The present results extend these earlier observations by examining suppression of hepatic glucose at a lower plasma insulin concentration (55 μU/ml). In these lower dose insulin clamp studies, a similar inhibition of hepatic glucose production was observed in uremic and control subjects (83 and 88%, respectively). These results indicate that within the physiological range of insulin concentrations the liver of uremic man retains normal sensitivity to insulin, at least with respect to suppression of glucose production.

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